





First report of *Economidichthys pygmaeus* (Holly, 1929) (Gobiidae) in Lake Lesser Prespa, Greece

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Abstract

Lake Lesser Prespa in Greece is part of the Prespa Lakes area which is a Ramsar site and a Special Protection Area due to its international importance for endemic biodiversity and migratory birds. Twenty fish taxa have been reported in that lake. From a field survey in July 2016, the species *Economidichthys pygmaeus* (Holly, 1929) (Gobiidae) was identified based on a molecular analysis and can be added to the list of fish species inhabiting Lake Lesser Prespa, which is the most northern location where this species has been found in Greece.

Keywords

Fish, goby, lentic waterbody, Greek Ramsar Wetland

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Introduction

The Prespa Lake basin is comprised of the interconnected Lake Great Prespa (also called Megali Prespa) and Lake Lesser Prespa (also called Mikri Prespa) forming one of the largest water bodies in the Balkan area (Crivelli and Catsadorakis 1997). Lake Lesser Prespa is a relatively shallow (maximum depth 8.4 m), eutrophic lake with intermittent tributaries in the catchment and has limited outflow towards the deep Lake Great Prespa (maximum depth 55 m) (Hollis and Stevenson 1977). In addition, both lakes extend across the national

borders of Greece, Albania, and North Macedonia, while the entire area forms the first transboundary protected area in the Balkans (Mitchell 2002–2022). The Prespa Lake area is of great ecological value, recognized as Wetland of International Importance under the Ramsar Convention, and is a Special Protection Area (Directive 79/409/EEC) in accordance with European Union legislation due to its endemic biodiversity and significance to migratory birds (Crivelli and Catsadorakis 1997). The region boasts the world's largest breeding colony of

the Dalmatian Pelicans (*Pelecanus crispus* Bruch, 1832, which numbered 1150–1530 pairs in 2008–2012, and several hundred breeding pairs of Great White Pelican (*Pelecanus onocrotalus* Linnaeus, 1758) (Catsadorakis et al. 2015). Furthermore, the fish fauna is characterized by high endemism (Crivelli et al. 1997a). In Lesser Prespa (hereafter, “Lesser Prespa” will denote only the Greek part of the lake), 20 fish taxa have been documented, of which four species (*Barbus prespensis* Karaman, 1924, *Chondrostoma prespensis* Karaman 1924, *Chalcalburnus belvica* Karaman, 1924 and *Cobitis meridionalis* Karaman, 1924), three subspecies (*Alburnoides bipunctatus prespensis* (Karaman, 1924), *Paraphoxinus epiroticus prespensis* (Karaman, 1924), and *Rutilus ohridanus prespensis* (Karaman, 1924)), and two hybrids, *Rutilusohridanus prespensis* × *C. belvica* and *C. belvica* × *Alburnoides bipunctatus prespensis* are endemic (Crivelli et al. 1997a, 1997b). The lake

is cyprinid-dominated, and the most valuable commercial species is *Cyprinus caprio* Linnaeus, 1758 (Crivelli 1997a; Bounas et al. 2021). The introduced *Cyprinus caprio*, *Pseudorasbora parva* Temminck & Schlegel, 1846, and *Lepomis gibbosus* Linnaeus, 1758 have been well established in the lake for years (Rosecchi et al. 1993; Bounas et al. 2021).

During a field course in 2016 in Lake Lesser Prespa, two small gobies were collected from a shallow-water site at the north part of the lake. Molecular analysis was required in order to accurately identify the species.

Methods

On 18 July 2016, two small fish were caught using a 0.5 mm mesh size hand net just before the beginning of the floating bridge towards the Island Agios Achilios (40°47'37.1"N, 021°04'26.4"E; Fig. 1) in Lake Lesser

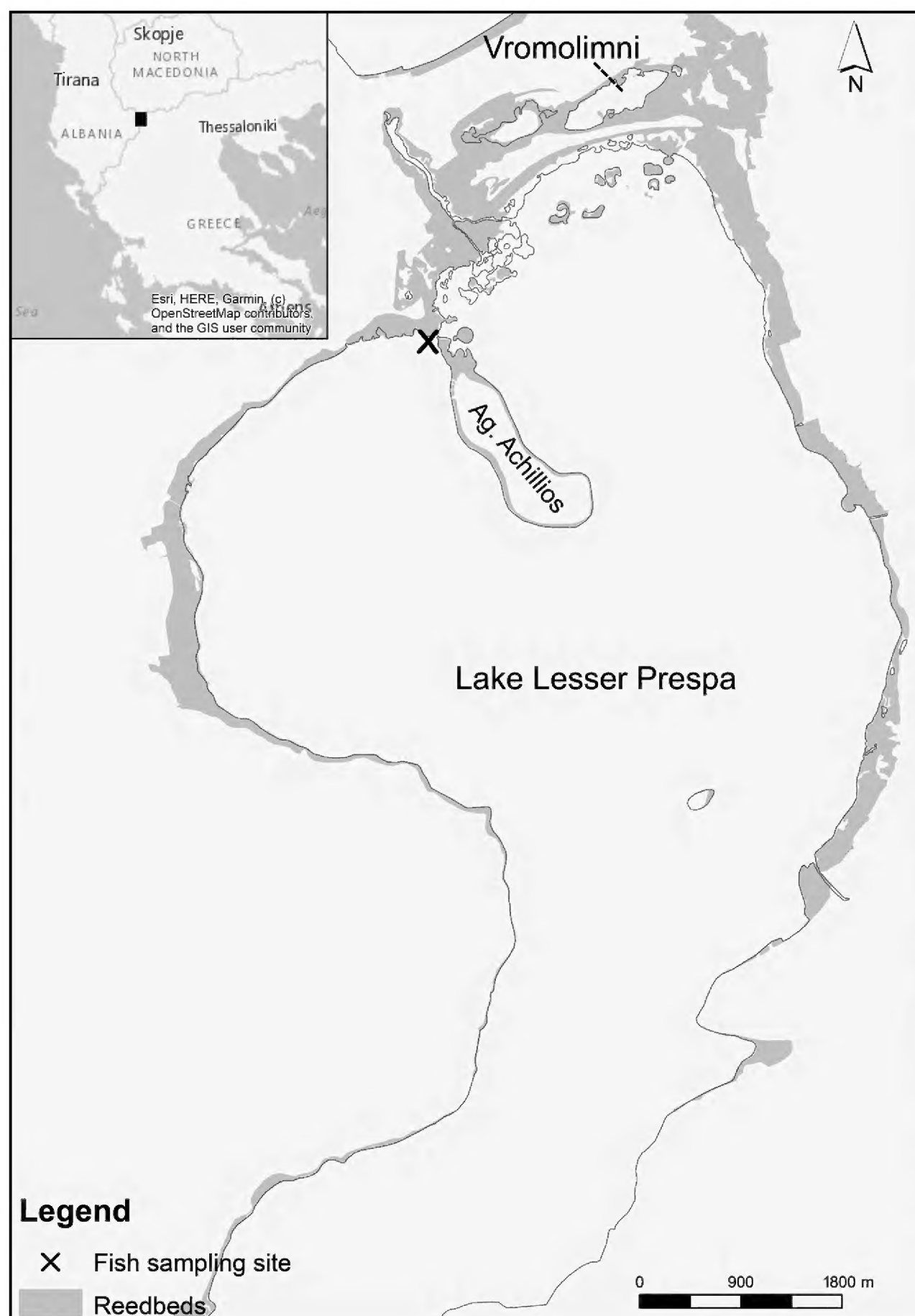


Figure 1. Location of Lake Lesser Prespa in the north-western Greece and the site where the sand goby *Economidichthys pygmaeus* was collected in July 2016 (indicated by the symbol ‘x’). Map credit: Yvon Verstijnen.

Prespa. The hand net was used to collect some parts of the observed macrophyte species while the two small fish were caught. The sampling site was a stagnant, shallow spot near the lake shore having high abundance in submerged macrophytes (Fig. 2). Both fish were later identified morphologically under a dissecting microscope as gobies (Fig. 3).

Given that Gobiidae have not been reported before in the Greek part of the Prespa lakes (Crivelli et al. 1997a) and the uncertainty and controversies on sand goby taxonomy based on morphology (Vanhove et al. 2012, 2016), the two specimens were subjected to a DNA analysis in the laboratory of Datura Molecular Solutions BV, Wageningen, the Netherlands.

DNA was extracted using the Qiagen DNeasy Blood and Tissue kit (Qiagen) and analysis of partial mitochondrial 16S ribosomal RNA sequences was performed. The 16S fragment was amplified using the forward primer 16Sar5' (5'-CGC CTG TTT ATC AAA AAC AT-3') and the reverse primer 16Sbr3' (5'-CCG GTY TGA ACT CAG ATC AYG T-3') (Palumbi 1996). The polymerase chain reactions (PCRs) were performed using Qiagen DNA polymerase. The PCR was done in a total volume of 25 µl: 2.5 µl 10× Qiagen buffer, 0.25 µl Qiagen Taq DNA Polymerase (5 U µl⁻¹), 0.7 µl MgCl₂ (25 mM), 2 µl dNTP (2.5 mM), 1 µl of each primer (10 pMol µl⁻¹), 14.6 µl MiliQ water, and 1 µl template. The PCR thermocycle profile used was: initial denaturation at 94 °C for 3 min, followed by 40 cycles (94 °C for 15 s, 52 °C for 30 s, and 72 °C for 40 s), and final extension of 5 min at 72 °C. The amplified 16S fragments were sequenced using a Sanger

Sequencing ABI3730XL sequencer (Life Technologies). Identities of the sequences were confirmed with BLAST searches in GenBank (NCBI). Additionally, associated sequences of related species were downloaded and compared in Geneious (Kearse et al. 2012).

Results

The obtained sequences (Genbank accession number MF182642) were matched with 100% identity and 93% coverage with the sequence of *Economidichthys pygmaeus* (Genbank accession number JN388374.1, isolate KPulA, location: Kalamas river delta, Greece). Therefore, the two gobies caught in Lesser Prespa were identified as *E. pygmaeus* (Fig. 3). This species is also known by the English common name Western Greece Goby (Barbieri et al. 2015).

New record. GREECE – **Prespa** • Lesser Prespa Lake; 40°47'37.1"N, 021°04'26.4"E; 853 m alt.; 18.XII.2016; F. van Oosterhout leg.; 0.5 mm mesh size hand net; 2 spec., MF182642.

Identification. Both fish were merely identified as *E. pygmaeus* based on the 16S fragment. The body of both gobies was elongated (up to 20 mm long for the specimen in Fig. 3) with crossing darker lateral midline marks and a rounded caudal fin. A dermal perianal organ was observed to some extent on the rear abdomen of both specimens, which is a unique feature of the gobies of the genus *Economidichthys* (Bianco et al. 1987; Economidis and Miller 1990).

Discussion

Economidichthys pygmaeus is listed as a protected species in Greece (Barbieri et al. 2015); however, the conservation status of *E. pygmaeus* is Least concern, meaning that it faces no critical threats (Economidis 2009).

Economidichthys pygmaeus is widespread in freshwaters in western Greece which include the Kalamas, Louros, Arachthos, and Acheloos rivers; the lakes Pamvotis, Trichonis, Lysimachia, Ziros, and Ozeros; Vlychos and Chiliadou springs, and the Mornos Delta (Miller 1990; Crivelli 2006; Barbieri et al. 2015; Fig. 4). Moreover, the species has been introduced to the Boeotian Cephissus, Lake Yliki in Central Greece, and Lake Taka in Peloponese (Barbieri et al. 2015; Fig. 4). According to Koutsikos et al. (2021), the suspected pathway of *E. pygmaeus* within the inland waters of Greece is unintentional or unknown but established where it is nonindigenous. The possible more widespread distribution area of this species in Greece is confirmed by the identification of *E. pygmaeus* at Lesser Prespa, which is about 100 km north-east of the boundary of the range so far known (Crivelli 2006; Barbieri et al. 2015).

A continuous population of *E. pygmaeus* appears to be present in Lake Great Prespa according to latest findings in 2017 and 2020 at the North Macedonian and



Figure 2. View of the sampling site of the two sand gobies *Economidichthys pygmaeus* at Lake Lesser Prespa in July 2016 (photo credit: Valentini Maliaka).

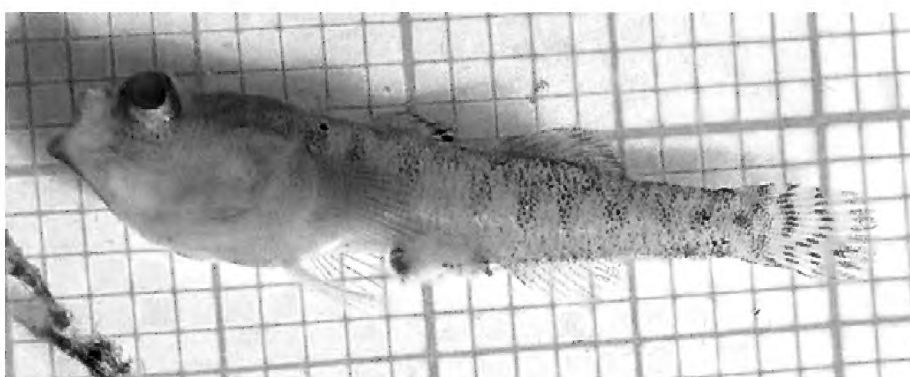


Figure 3. A specimen of *Economidichthys pygmaeus* from Lake Lesser Prespa (1 × 1mm grid paper in the background) (Photo credits: Valentini Maliaka).

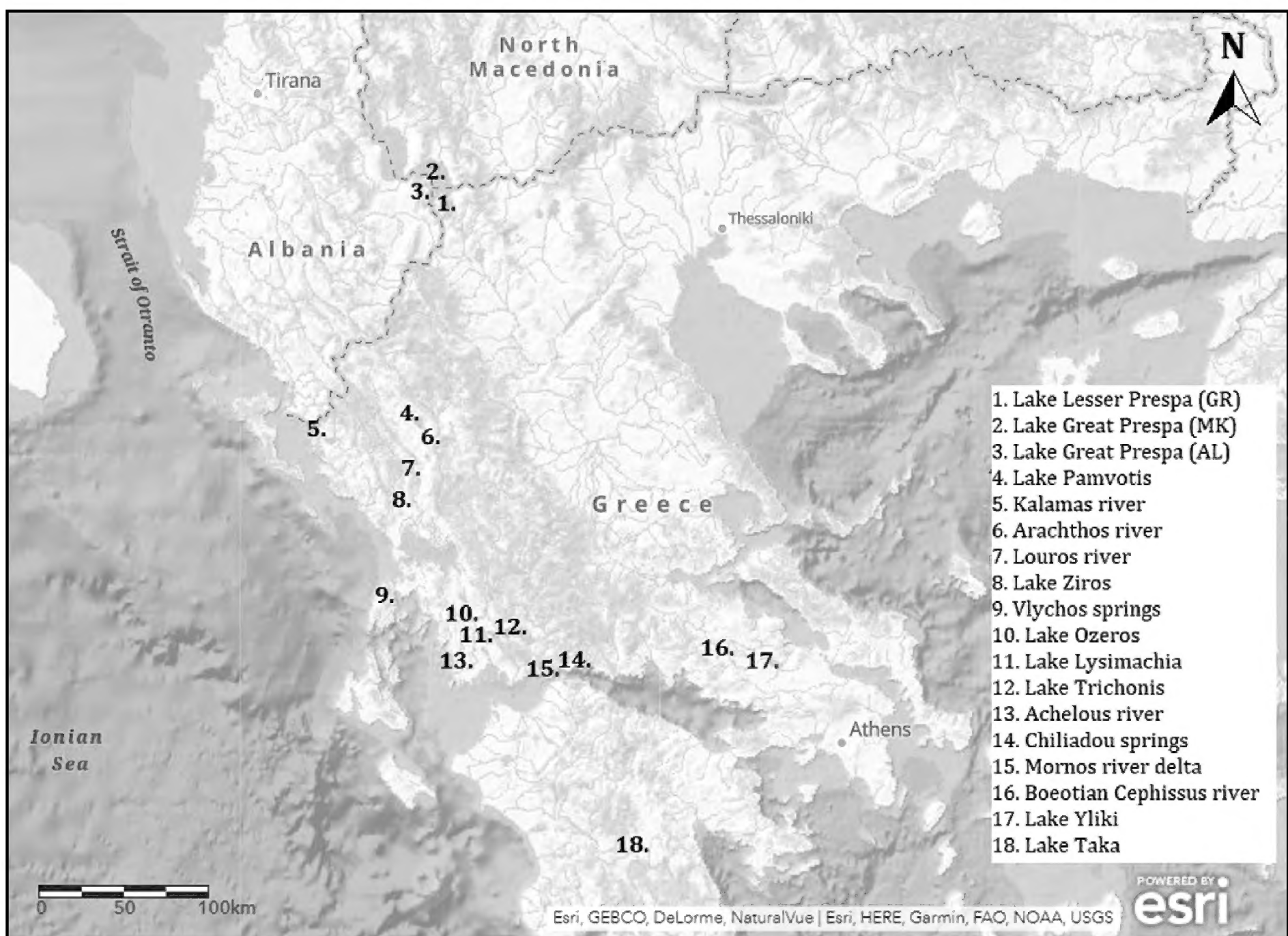


Figure 4. Distribution of *Economidichthys pygmaeus* in Greece and the transboundary Lake Great Prespa, which is shared among North Macedonia, Albania, and Greece. Map Source: Esri, GEBCO, NOAA, National Geographic, De Lorme, HERE, Geonames.org, and other contributors.

Albanian part of the lake (Trajchevski et al. 2020; Fig. 4). Ten specimens, which were caught for the first time at Lake Great Prespa (North Macedonia) in 25–31 July 2017, were identified as *E. pygmaeus* using the identification key to gobies by Kottelat and Freyhof (2007) and the key to Greek freshwater gobies by Economidis and Miller (1990) (Trajchevski et al. 2020). These sand gobies had different sizes and were caught at four distant locations which may indicate that there has been an established population of *E. pygmaeus* in Lake Great Prespa (Trajchevski et al. 2020). The four sampling locations in Lake Great Prespa were 14.3 km to 24.7 km north-west and 18.6 km north-east of where we found our two *E. pygmaeus* species Lake Lesser Prespa. Another 16 specimens of *E. pygmaeus* were later caught at the Albanian part of Lake Great Prespa on the 13–20 August 2020, which represents the first records of this species in this part of the lake (Shumka et al. 2020). The sampling location of these sand gobies was 16.1 km north-west of our sampling site.

The above-mentioned records of *E. pygmaeus* in Lake Great Prespa in 2017 and 2020 (Trajchevski et al. 2020) and Lesser Prespa in 2016 (our study) give valuable information on the biogeographical distribution of this species in the transboundary Prespa area. The Prespa lakes area can provide a suitable habitat to *E. pygmaeus*

that uses reed cavities and *Phragmites* plants as spawning sites (Crivelli 2006; Vanhove et al. 2012). As Dalmatian Pelicans frequently fly from Lesser Prespa to the waters in the region where *E. pygmaeus* has been found, such as Lake Great Prespa and Lake Pamvotis (Shumka et al. 2020; Trajchevski et al. 2020; BirdLife International 2022; Gkenas et al. 2012), passive dispersal via an avian vector cannot be excluded, although this has been challenged (Jubb 1964). An unintentional introduction of this species through fish stocking at the lake (Crivelli et al. 1997a) could also explain the presence of *E. pygmaeus*. Two reasons why *E. pygmaeus* has not been discovered until now in Lesser Prespa could be the use of rather large mesh in nets (>10mm) to determine the fish stocks (Crivelli et al. 1997a) and not looking for small-bodied gobies in their preferred habitat. The presence of *E. pygmaeus* in Lesser Prespa suggests that a more thorough investigation is warranted for potentially other sand gobies in the lake.

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Authors' Contributions

Conceptualization: FvO. Data curation: FvO, ML. Formal analysis: FvO, KvB. Funding acquisition: ML. Investigation: FvO. Methodology: KvB. Project administration: ML, VM. Supervision: ML. Validation: KvB. Visualization: VM. Writing – original draft: FvO, VM. Writing – review and editing: ML.

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